AUTOMATIC DEVICE FOR DISPENSING SAMPLES WITH A VIEW TO
IMPLEMENTING CHEMICAL OR BIOLOGICAL REACTIONS IN LIQUID
MEDIUM, FOR USE IN CHEMICAL OR BIOLOGICAL REACTIONS

5          The present invention relates to an automatic
device for producing a plurality of reaction samples
from several constituents for implementing chemical or
biological reactions in liquid medium, in particular
the dosing of at least one particular constituent in a
10   biological specimen.
           Numerous   methods   have   been   developed   for
identifying,  detecting  or  quantifying  analytes  in
chemical or biological constituents.
           These   methods   are   usually   based   on   the
15   formation  of  complexes  by  affinity  reaction  between
members of a specific binding pair.
           Reactions,  of  ligand/receptor  type,  result  for
example  from  interactions  between  an  antigen  and  a
specific  antibody,  from  hybridization  between  two
20   complementary   nucleic   acid   sequences   or   from   a
phenomenon of recognition between the binding site of a
protein,  for  example  an  enzyme,  hormone,  or  other
biological   entity,   and   its   ligand,   substrate   or
receptor.
25         The  formation  of  an  affinity  complex  makes  it
possible  to  reveal  the  presence  of  the  sough-after
analyte  in  the  sample. This  analyte  may  possibly  be
quantified,  if  it  is  possible  to  separate  the  complexed
forms  from  those  remaining  in  the  free  state,  or  to
30   measure the degree of occupancy of the specific ligands
of the analyte.
           This   type   of   method   for   detecting   and
quantifying   an   analyte   present   in   a   constituent,
sometimes  in  trace  quantities,  offers  great  benefit  for
35   research   or   analysis   laboratories,   in   particular
laboratories for clinical or biological analysis.
           However,  for  routine  use,  the  methods  must  be
able  to  be  applied  simultaneously  to  a  large  number  of

constituents. Furthermore, for one and the same constituent, it is often necessary to carry out several tests so as to reveal the formation of various complexes, to dose several analytes of this
5   constituent.

Moreover, in the field of genetic analysis, the technique of genetic amplification dubbed "Polymerase Chain Reaction" (PCR) makes it possible in particular, on the basis of DNA specimens, to identify DNA marker
10  sequences for genetic diseases, to identify and isolate DNA sequences with a view to cloning or else to quantitatively dose particular DNA sequences.

This technique makes it possible to amplify in a sample a particular DNA sequence with a view to
15  analyzing it.

The reaction samples employed in this PCR technique consist of three different constituents: the biological specimen containing the DNA, the specific primers (oligonucleotides) for the sequence to be
20  analyzed and the PCR mix comprising the polymerase and the nucleotides.

The implementation of such a technique on a large number of different DNA specimens, then requires the implementation of a large number of reaction
25  samples produced by cross-combining at least two series of numerous constituents.

More generally, the characterization of a large number of chemical or biological properties over a wide selection of different constituents requires the
30  production of a large number of samples from at least two series of numerous different constituents.

In order to lower the costs of implementing such characterization techniques, it is currently advisable to reduce the volume of the constituents used
35  as far as possible.

In particular, in the field of genetic analysis, the current trend is to reduce the volumes of

constituents handled by a factor of 1000, thereby taking them from a few microliters to a few nanoliters.

Thus, currently, the technology of DNA microchips is being developed, making it possible to
5  carry out simultaneous reactions of a large number of reagents with one and the same biological specimen on a miniaturized sample support.

However, such a technology does not make it possible to produce a large number of reaction samples
10 automatically from at least two series of different constituents.

As compared with the state of the art, the present invention proposes a novel automatic device making it possible to carry out the controlled
15 dispensing of nanovolumes of liquid constituents into a plate containing a large number of miniaturized cavities so as to perform multiple mixings in nanovolumes in order to produce reaction samples.

More particularly, according to the invention,
20 there is provided an automatic device for producing a plurality of reaction samples from several constituents for implementing chemical or biological reactions in liquid medium, in particular the dosing of at least one particular constituent or analyte in a biological
25 specimen, such a device comprising:
- a first supply plate, in particular a removable plate of the microplate type, comprising N receptacles each intended to contain a constituent,
- a second supply plate, in particular a removable
30 plate of the microplate type, comprising M receptacles each intended to contain a constituent,
- a removable plate for samples, comprising a plurality of cavities arranged in the form of an array comprising at least N rows and at least M columns, each
35 cavity exhibiting a volume of the order of a few tens of nanoliters, and being intended to contain a mixture of constituents originating from the first and second supply plates,

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- a piezoelectric micropipette able to sample a determined amount of constituent and to deliver drops of volume of the order of a nanoliter,

- means for displacing the piezoelectric
5  micropipette along at least two perpendicular axes Y, Z so that it can sample from each filled receptacle of the first and second supply plates, the determined amount of a constituent, and

- means of relative displacement of the
10  piezoelectric micropipette and of the sample plate, which are associated with means for triggering discharge of the micropipette in such a way that the latter delivers at least one drop of constituent into each cavity of the sample plate.

15      The expressions N receptacles, and M receptacles of the first and second supply plates are understood to mean the receptacles intended to be actually filled with a constituent. It should of course be understood that these first and second supply plates
20  can contain a number of receptacles greater than N respectively M, with a certain number of unused useful receptacles.

        Thus, by virtue of the combining of the aforesaid means of the device according to the
25  invention, it is possible to produce thousands of different samples per hour in volumes of a few nanoliters with a view to performing chemical or biological reactions.

        By virtue of the miniaturization of the sample
30  volumes, the cost of said chemical or biological reactions is thus reduced.

        Apart from the fact that the automation of the device makes it possible to obtain a high rate of production of sample plates, it makes it possible to
35  comply with proper safety and hygiene conditions.

        According to other advantageous and nonlimiting characteristics of the device according to the invention:

- The relative displacement of the piezoelectric micropipette and of the sample plate is continuous and the discharge triggering means are able to trigger discharges of the micropipette at regular time intervals as a function of the constant speed of relative displacement of said micropipette and of the sample plate, independently of the presence or otherwise of a cavity of the sample plate in line with the micropipette.

- The means of relative displacement are means for advancing the sample plate along an axis X parallel to the rows of the arrays of cavities and/or along an axis Y parallel to the columns of the arrays of cavities, the piezoelectric micropipette remaining stationary above the sample plate during the filling of said cavities.

- The means of relative displacement are means for advancing the micropipette along axes X and/or Y respectively parallel to the rows and to the columns of the arrays of cavities of the sample plate which remains stationary beneath the micropipette during the filling of said cavities.

- The advancing means comprise a stepper or DC motor, and the discharge triggering means comprise a counter of the steps of the motor able to send a discharge triggering signal every $N_1$ steps.

- The piezoelectric micropipette is able to count the number of drops which it delivers and to stop discharging after a determined number of drops delivered.

- The device comprises a refrigerating tray supporting the sample plate.

- There is provided at least one optical system such as the emission/reception of a laser carpet, in line with the sample plate, able to count the number of drops delivered with each discharge of the piezoelectric micropipette, and to transmit this number to a coordination device so that an order for a second

pass in line with a cavity or several cavities is sent
to the micropipette when a discrepancy is noted between
the counted number of drops discharged and the
designated theoretical number of drops.

5      -      The device comprises another piezoelectric
micropipette identical to the first, the two
micropipettes operating alternately.
       -      The device comprises an automatic washing station
associated with each piezoelectric micropipette
10     ensuring the decontamination of the latter.
       -      Each washing station comprises means for filling
the piezoelectric micropipette with a carrier liquid
and optical means for verifying the proper filling of
said micropipette.
15     -      Each piezoelectric micropipette comprises two
conducting parts separated by a nonconducting material,
which are electrically linked at the upper part to an
electrical system, so that when the orifice of the
piezoelectric micropipette comes into contact with a
20     constituent of a receptacle of the first or of the
second supply plate, it brings about closure of the
electrical circuit formed by the two electrically
linked conducting parts of said micropipette, which
commands the stoppage of the vertical displacement of
25     the micropipette.

       The description which follows with regard to
the appended drawings, given by way of nonlimiting
examples, will clearly elucidate that of which the
invention consists and how it may be embodied.
30     _Brief Description of the Drawings_
       In the appended drawings:
       -      Figure 1 is a diagrammatic plan view of an
embodiment of the automatic device according to the
invention,
       -      Figure 2 is a partial cross-sectional view of
35     the sample plate of the automatic device according to
the invention, and
       -      Figure 3 is a diagrammatic view of a detail of
Figure 2.

EMBODIMENTS OF THE INVENTION

Represented in Figure 1 is an automatic device 100 for producing a plurality of samples from several constituents for implementing chemical or biological reactions in liquid medium, here in particular the gene

5    amplification technique (PCR technique).

This device 100 comprises a first supply plate 110, here a removable plate of the microplate type which comprises 96 wells or cavities of a capacity of the order of from 100 to 300 microliters, each intended

10   to contain a constituent, here a DNA specimen.

Advantageously, each receptacle contains a different constituent.

It furthermore comprises a second supply plate 120, here identical to the first plate, that is to say

15   a removable plate of the microplate type comprising 96 wells or cavities, each intended to contain a constituent. In the particular case described, the constituents placed in the cavities of the second supply plate 120 are primers which can be fluorescent,

20   consisting of specific oligonucleotides for the particular DNA sequences to be amplified.

It is also advantageous for each receptacle of the second supply plate 120 to contain a different constituent.

25   There is provided a removable sample plate 130, which comprises a plurality of cavities 134 (see Figure 2) arranged in the form of an array 131, here a square arrays which comprises 100 rows and 100 columns for example.

30   Each cavity 134 of the sample plate 130 exhibits a volume of the order of a few tens of nanoliters, here of the order of 60 nanoliters, and is intended to contain a mixture of constituents originating from the first and second supply plates

35   110, 120.

More particularly, the arrays 131 of the sample plate 130 here exhibits a length and a width of around 5.5 cm, the cavities 134 are spaced apart successively

in a row by a length $d_2$ of the order of 150 µm (see
Figure 3). They exhibit a width $d_1$ of around 400 µm for
a depth $h$ (see Figure 2) of the order of 400-500 µm.

The sample plate 130 consists of a glass
support wafer 131b, transparent to UV rays, onto which
is chemically bonded ("anodic bonding") a silicon grid
131a whose holes correspond to the cavities 134 of the
sample plate. This silicon grid 131a is made from a
silicon wafer masked and drilled by photolithography.

The first supply plate 110 and the second
supply plate 120 are disposed on either side of the
sample plate 130 along an axis X, on a bench 101.

As will be described in greater detail later,
this bench 101 is here able to move in translation
along the X axis.

It is interesting to note that according to an
advantageous characteristic of the device 100, the
sample plate 130 is disposed on a refrigerating support
(not represented), so that the small amount of product
which arrives in each of the cavities of the arrays 131
of the sample plate 132 is cooled and possibly frozen.

The evaporation of the product placed in each
of the cavities of the sample plate is thus limited.

According to the example represented, there is
provided in proximity to the sample plate 130 on the
bench 101, two vessels 151, 152 disposed symmetrically
on either side of the X axis of the bench 101 and
intended to each contain another constituent, here the
PCR mix mixture which contains a polymerase and the
nucleotides. These vessels 151, 152 are able to contain
100 to 500 microliters of product.

The device 100 comprises a first piezoelectric
micropipette 141 able to sample a determined amount of
constituent and to deliver drops of constituent of a
volume of the order of a nanoliter.

The amount sampled by the piezoelectric
micropipette 141 is of the order of a microliter. Of

course, provision may be made for it to sample several microliters.

This micropipette 141 is mounted on a motorized rig 103 of the device 100 so as to be able to move along a Y axis transverse to the X axis situated in the same plane as the latter, and along a Z axis perpendicular to the X and Y axes so that it can sample from each filled receptacle of the first and second supply plates 110, 120 the determined amount of a constituent, as will be described later.

Generally, motor means may be provided in the device 100 according to the invention, so as to move the piezoelectric micropipette 141 along the perpendicular X, Y, Z axes.

In the example represented, there is also provided a second piezoelectric micropipette 142 identical to the first piezoelectric micropipette 141 disposed symmetrically with the first relative to the X axis so that the orifices of the micropipettes are perfectly aligned on one and the same Y axis.

The second piezoelectric micropipette 142 is mounted on a motorized rig 102 in such a way as to be able to move along the perpendicular Y and Z axes. Here again, provision may be made for it to be movable along the X axis in a variant embodiment (not represented).

The two piezoelectric micropipettes 141, 142 are able to operate alternately so as to fill the cavities disposed in rows and columns of the arrays 131 of the sample plate 130.

There are provided means of relative displacement of each piezoelectric micropipette 141, 142 and of the sample plate 130, which are associated with means for triggering discharge of each micropipette 141, 142 in such a way that the latter delivers at least one drop of constituent into each cavity 134 of the sample plate 130.

Here, advantageously, the relative displacement of the piezoelectric micropipette 141, 142 and of the

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sample plate 130 is continuous and the discharge
triggering means are able to trigger discharges of the
corresponding piezoelectric micropipette 141, 142 at
regular time intervals as a function of the constant

5    speed of relative displacement of the micropipette 141,
142 and of the sample plate 130, independently of the
presence or otherwise of a cavity 134 of the sample
plate 130 in line with the micropipette 141, 142.

        To do this, there is provision in order to fill

10   the row-wise cavities of the arrays 131 of the sample
plate 130, according to the embodiment represented in
Figure 1, for the means of relative displacement to
comprise means for advancing the sample plate 130 along
the X axis parallel to the rows of the arrays 131 of

15   cavities 134, the corresponding piezoelectric
micropipette 141, 142 remaining stationary above the
sample plate 130 during the filling of the cavities of
the plate.

        Said advancing means comprise a stepper or DC

20   motor, and the discharge triggering means comprise a
counter of steps of the motor able to send an external
electrical discharge triggering signal every $N_1$ steps.

        More particularly, according to the example
represented, each piezoelectric micropipette 141, 142

25   advantageously exhibits an ejection frequency of 1000
hertz thereby enabling it to deliver 10 drops of 1
nanoliter in $10^{-2}$ seconds.

        It is interesting to stress that the ejection
frequency of the piezoelectric micropipette must not be

30   too high so as to avoid any risk of emptying the latter
(by cavitation).

        The ejection frequency is then chosen in an
optimum manner so that it is high enough while avoiding
the risks of emptying the micropipette during action.

35       In the typical case, it is envisaged that with
each pass of the piezoelectric pipette above a cavity,
the latter shall deliver 10 drops of constituent of 1

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nanoliter into the corresponding cavity 134 of the arrays 131 of the sample plate 130.

To do this, the piezoelectric micropipette 141, 142 discharges 10 drops over a distance $d_1$ of 100 μm in the vicinity of each central axis of each cavity 134, as is represented more particularly in Figure 3.

The piezoelectric pipette then delivers 10 drops of 1 nanoliter in $10^{-2}$ seconds over a distance of 100 μm in the vicinity of each center of each cavity 134 of the arrays 131 of the supply plate, thereby making it possible to determine the continuous speed of advance of 10 mm/s of the arrays 131 of the sample plate with respect to the stationary piezoelectric pipette.

Advantageously, each piezoelectric micropipette 141, 142 is such that it is able to count the number of drops which it delivers and to stop after a determined number of drops, fixed beforehand.

In the present case, it thus stops after 10 drops of constituent delivered.

A stepper or DC motor (not represented in the figure) makes it possible for the bench 101 on which the sample plate sits to be moved continuously, for example along the arrow F as represented in Figure 3 at a constant speed of 10 mm/s. This motor counts its steps and after a certain number of steps $N_1$ corresponding to the distance traveled $d_1 + d_2$, it sends an order (via an external electrical signal) to the relevant piezoelectric micropipette 141, 142, triggering discharge of the 10 drops of constituent, while this pipette is in principle in proximity to a center of a cavity 134. The discharging of the micropipette proceeds over a distance $d_3$ equal to 100 μm after which the micropipette which has in principle counted 10 drops of constituent, stops delivering the constituent of the product.

The stepper or DC motor which continues to count its steps, advances the sample plate by a

distance $d_4$ equal to around 500 μm before sending a new
discharge triggering order to the piezoelectric
micropipette, the latter normally being located in
proximity to the center of the next cavity 134 in the
same row, and so on and so forth.

It should be noted that these discharge
triggering means are independent of the fact that a
cavity of the sample plate is located in line with the
orifice of the piezoelectric micropipette, but are
dependent on the speed of relative advance of the
sample plate and of the micropipette, so that when the
micropipette triggers a discharge its orifice is
located in proximity to a center of a cavity.

Of course, according to a variant (not
represented), means for displacing the micropipette
relative to the sample plate, the latter remaining
stationary, may be provided so as to fill the row-wise
cavities of the sample array.

These means for displacing the micropipette can
be a stepper or DC motor which counts its steps and
which triggers the discharging of the micropipette when
the end of the latter passes in the vicinity of a
center of a cavity of the sample plate, as described
earlier.

Such a means for displacing the micropipette
along the Y axis parallel to the columns of the array
of the sample plate is used according to the example
described to fill the cavities of the array column-
wise.

On either side of the sample plate 130 disposed
on the bench 101, the device 100 comprises means for
controlling the number of drops delivered into each
cavity, here a system for emitting/receiving a laser
carpet 161, 162 whose beam is aligned on the axis of
displacement of the micropipettes.

The system for emitting/receiving the laser
carpet 161, 162 is able to count up and transmit the
number of drops delivered into each cavity by the

piezoelectric micropipette, to a coordination device, a
comparison between the number delivered and the
designated theoretical number (here the number 10) is
performed by the coordination device and when a
discrepancy is noted between the number actually
delivered and the designated theoretical number, the
coordination device sends an order for a second pass of
the piezoelectric micropipette over the relevant cavity
or cavities so as to deliver the missing drop or drops.
If the discrepancy noted is greater than a given
threshold, the system is able to record this
discrepancy in a computer file.

          As shown in Figure 1, an automatic washing
station 170 is provided in the device 100 for each
piezoelectric micropipette 141, 142. Each micropipette
having its own washing station as close as possible to
the relevant pipette. These two washing stations 170
are disposed symmetrically with respect to the X axis
on either side of the bench 101 and are aligned on the
axis of displacement of the micropipettes.

          Each automatic washing station 170 is such that
it makes it possible between each filled row or column
of the array, to wash the micropipette with Javel water
for example, so as to decontaminate it, and to fill it
with a water-immiscible carrier liquid, octane for
example. Advantageously, there is provided at each
washing station 170, an optical means, such as a laser
beam 171 which makes it possible to control the proper
filling of the micropipette with the carrier liquid.

          Furthermore, the device 100 comprises an
automatic system for removing and fitting covers 180
which is fixed on a motorized rig 105 above the bench
101, outside the second supply plate 120 (with respect
to the sample plate). This system 180 for removing and
fitting covers here comprises three suckers 181, 182,
183 disposed at the end of arms extending radially from
a central point, and is able to move along the Z axis
so as to seize a cover for example a cover 133 of the

sample plate, which cover is made of a glass which is
transparent to UV rays, and when the sample plate 130
is placed beneath said system 180, to fit this cover
133 on the filled sample plate 130 so as to close it.
5   The system 180 for removing and fitting covers is also
intended for taking off the covers (not represented) of
the first and second supply plates before initiating
the procedure for filling the array of the sample
plate.

10        Opposite the automatic system 180 for removing
and fitting covers, there is provided an automatic
system 190 for fitting a seal to the sample plate 130
before setting the cover in place. This system 190 is
mounted on a rig 104 above the bench 101 and is
15  motorized in such a way as to move along the Z and Y
axes so that, when the sample plate 130 is situated
beneath this system 190, by advancing along the X axis,
the displacement along the Y axis of the seal fitting
system 190 makes it possible to place a circular seal
20  on the sample plate 130. In the case where the latitude
of displacement of the bench 101 is limited along the X
axis, there may be provided at the level of the
automatic seal fitting system 190, another cover
removal and fitting system identical to that described
25  earlier and intended for taking off and replacing the
cover of the supply plate 110 situated in proximity
thereto.

          According to a particularly advantageous
characteristic, each piezoelectric pipette 141, 142 of
30  the device according to the invention comprises two
conducting parts separated by a nonconducting material,
which are linked to an electrical system, so that when
the orifice of the micropipette comes into contact with
a constituent of a receptacle of the first or the
35  second supply plate, it brings about closure of the
electrical circuit, which causes the former to stop in
the vicinity of the surface of the liquid.

The micropipette thus comprises a level detector system so that it does not sink too far into each receptacle of each supply plate.

The manner of operation of the device 100 represented in Figure 1 is as follows.

Firstly, the bench 101 moves along the X axis so as to bring the supply plate 110 beneath a cover removal and fitting system 180, so that the latter takes the cover off this plate, which remains suspended by the suckers above the bench.

Starting from the rest position represented in Figure 1 in which it is assumed that each of the micropipettes 141, 142 is filled correctly with a carrier liquid, the first micropipette 141 moves along the Y axis so as to come above the bench 101 and the first supply plate 110 moves along the X axis over the bench 101 so that the orifice of the micropipette is located in line with the first receptacle for example.

The first micropipette descends along the Z axis so as to sample a dose of around 1 microliter of the constituent in the receptacle, then rises back into position just above the supply plate 110. The proper priming of the micropipette is then verified by redispensing a few drops of the constituent into the receptacle.

The sample plate 130 then comes into position beneath the micropipette so that its orifice is placed in line with the first cavity of the first row of the array 131 of the sample plate 130.

The stepper or DC motor then displaces the bench 101 so as to continuously advance the array 131 of the sample plate along the X axis at the speed of 10 mm/s (as described earlier) the micropipette discharging at regular time intervals 10 drops of 1 nanoliter at a frequency of 1000 hertz, into each of the cavities of the first row of the array 131 of the sample plate 130.

With each discharge of the micropipette, the system for emitting/receiving the laser carpet 161, 162 counts the drops delivered.

When the micropipette 141 reaches the end of
5  the row, the sample plate 130 changes direction of travel so that the cavities of the array 131 which are already filled pass beneath the micropipette again so that the latter can optionally deliver additional drops into certain cavities which comprise fewer than 10
10  drops, the correction order having been obtained by virtue of the system for emitting/receiving the laser carpet 161, 162 as described earlier.

The micropipette 141 having performed a second pass then returns by moving along the Y axis to the
15  level of the automatic washing station 170 situated alongside it, so as to be washed with the Javel water (for example) and refilled with the carrier liquid. It then positions itself on standby.

The second micropipette 142 comes into action
20  when the first micropipette 141 has terminated its second pass above the first row of the sample plate 130.

The micropipette 142 performs its translational motion along the Y axis while the first supply plate
25  110 is moving along the X axis so that the micropipette 142 comes into position in line with the second cavity of the first supply plate 110. It then moves along the Z axis so as to sample a determined amount of the order of 1 microliter of the constituent located in this
30  cavity.

When the second micropipette 142 is filled with the second constituent, it rises slightly along the Z axis so as to verify the priming thereof. It also moves along the Y axis while the bench 101 is bringing the
35  sample plate 130 along the X axis until it is beneath the micropipette 142, so that its orifice is placed in line with the first cavity of the second row of the array 131. The latter advances under the action of the

motor, as described earlier, and the micropipette 142
discharges, at regular time intervals, 10 drops at a
frequency of 1000 hertz into each of the cavities of
the second row of the array 131 of the sample plate 130
5    when the orifice of the micropipette passes in the
vicinity of the center of each of the cavities.

In the same way, having reached the end of the
row, the sample plate 130 changes direction of travel
and the array passes beneath the micropipette 142 again
10   so that it can perform corrections of discharge into
certain cavities, the order having been obtained by
virtue of the system for emitting/receiving the laser
carpet 161, 162.

And so on, alternately the micropipettes 141,
15   142 fill the row-wise cavities of the array 131 of the
sample plate 132 with the various constituents
contained in the receptacles of the first supply plate
110.

Between each sample plate dispensing cycle, the
20   micropipettes are washed in their washing station 170
and are filled with the carrier liquid, their priming
having been controlled by virtue of the corresponding
laser beam device associated with each of the washing
stations 170.

25   The bench 101 moves along the X axis so that
the cover removal and fitting system 180 refits the
cover onto the supply plate 110 and takes the cover off
the second supply plate 120.

Then comes the column-wise filling of the
30   cavities 134 of the array 131 of the sample plate 130,
with the constituents contained in the receptacles of
the second supply plate 120 of the device 100.

The micropipettes 141, 142 also operate
alternately every other column. Only the filling of a
35   column of the array of the sample plate will thus be
described hereinafter.

The micropipette 141 filled with the
appropriate carrier liquid moves along the Y axis so as

to be placed above the bench 101 while the second
supply plate 120 moves along the X axis until it comes
beneath the orifice of the micropipette so that the
latter can sample an amount of around 1 microliter of
5    the constituent of the first receptacle of the second
supply plate. Priming is verified as described earlier.

The micropipette 141 then moves along the Y
axis while the bench 101 moves along the X axis so that
the orifice of the micropipette is located above the
10   first cavity of the first column of the array 131 of
the sample plate 132.

The micropipette 141 then moves at constant
speed of around 10 mm/s along the Y axis along the
first column of the array, the motion of the
15   translation of the micropipette being provided by a
stepper or DC motor.

The micropipette 141 discharges, at regular
time intervals, 10 drops at a frequency of 1000 hertz
when its orifice passes in the vicinity of the center
20   of each of the cavities of the first column of the
array.

With each discharge, the system for
emitting/receiving the laser carpet 161, 162 controls
the number of drops delivered into the corresponding
25   cavity.

Having reached the end of the column, the
micropipette changes direction of travel and passes
back above the cavities it has just filled so as to
perform any corrections for which the order has been
30   obtained by virtue of the system for emitting/receiving
the laser carpet 161, 162.

Next, the micropipette 141 returns to the
washing station by moving along the Y axis so as to be
washed and refilled with the carrier liquid before
35   positioning itself on standby.

The second micropipette 142 comes into action
when the first micropipette 141 has terminated its
second pass. It then fills the cavities of the second

column with doses of 10 nanoliters of the constituent contained in the second receptacle of the second supply plate.

Thus, the columns and the rows of the array 131
5   of the sample plate 130 are filled with the various constituents of the first and second supply plates 110, 120.

The bench 101 moves along the X axis so as to position the second plate 120 beneath the system with
10  suckers 180 so that the latter replace the cover on said plate.

Lastly, comes the filling of the cavities 134 of the array 131 of the sample plate 130 with the PCR mix located in the vessels 151.

15      To do this, the dispensing method is identical to the filling of the rows of the array, as described earlier, the two micropipettes operating alternately.

Each micropipette 141, 142 coming firstly to the level of the bench 101 so as to sample a dose of
20  the constituent located in the vessel 151, 152, then the array 131 of the sample plate comes beneath the orifice of the micropipette and advances at constant speed and with each pass of the center of a cavity, the micropipette delivers 10 drops of the corresponding
25  constituent into the cavity.

Thus, the sample plate 130 is completely filled with two series of different constituents and a common constituent: a first series of constituents originating from the first supply plate corresponding to a series
30  of DNA specimens, a second series of constituents originating from the second supply plate corresponding to a series of specific primers for particular DNA sequences, and a third common constituent, the PCR mix.

The overall volume of these three constituents
35  in each cavity 134 of the array 131 of the sample plate 130, is of the order of 30 nanoliters.

As we have already explained, it is advantageous for the sample plate 132 to be disposed on

a refrigerating system so that the amount of constituent arriving in a cavity of the array is cooled. The problems of the evaporation of the very small amounts dispensed into the cavities is thus limited.

When the sample plate 130 is entirely filled, it moves along the X axis to the level of the seal fitting system 190, which descends along the Z axis to the level of the sample plate and moves along the Y axis while the sample plate 130 moves along the X axis, in such a way as to fit a circular seal onto the sample plate 130 around the array of cavities.

Next, the filled sample plate bearing its peripheral seal moves along the X axis so as to be placed beneath the cover removal and fitting system 180, which previously grabbed a cover 133 of the sample plate 130 using its suckers, and holds this cover above the bench 101.

It then receives the cover 133, made of a glass transparent to UV rays, on the seal which it bears.

The sample plate 130 is then ready to undergo the subsequent treatments so as here to implement the PCR technique.

Of course, the device 100 according to the invention makes it possible to fill the sample plate with all kinds of constituents with a view to implementing other chemical or biological reactions in liquid medium.

The device 100 according to the invention exhibits the following advantages.

- It is fully automated and makes it possible in a very short time of close to one hour to fill a sample plate comprising 10,000 cavities so as to produce 10,000 different samples of a volume of a few tens of nanoliters.

This is particularly advantageous in the field of genetic analysis where one seeks to limit the reaction volumes used. The device is also particularly

advantageous insofar as it produces N × M reaction samples from only N + M specimens.

- The sample plate as described according to the invention is removable and reusable after washing.

5 - The device 100 makes it possible to produce a sample plate very hygienically, thus making it possible to avoid contamination. Such a device also makes it possible to produce reaction samples very safely from dangerous constituents.

10 - Lastly, the device 100 is particularly advantageous insofar as it makes it possible to prepare reaction mixtures in a leaktight plate which may subsequently (after possible storage) be sent to an oven so as to undergo temperature cycles therein (for

15 example for implementing PCR reactions).

Of course, the present invention is in no way limited to the embodiment described and represented, but the person skilled in the art will be able to make any variations thereto in accordance with the spirit

20 thereof.

In particular, there may be provision for the array of the sample plate not to be a square array but an array comprising at least N rows corresponding to the N filled receptacles of the first filled supply

25 plate and at least M columns corresponding to the M filled receptacles of the second supply plate.

The first and second supply plates may be simple test-tube support insofar as one seeks to store a larger amount of product than that which can be

30 stored in the microwells of microplate-type plates.

Provision may be made for the device according to the invention to comprise a number greater than 2 of piezoelectric micropipettes operating alternately in a synchronized manner. Such a device can also comprise a

35 larger number (greater than 2) of supply plates, so as to prepare a sample plate from a larger number of series of constituents.